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THERMOLABILITY OF SO-CALLED SYPHILITIC ANTIBODY

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It is well known that unheated serums frequently give a stronger positive result with the Wassermann test than they do after they have been heated to 56 C. for 30 minutes. In order to avoid loss of complement-binding power, and for other reasons, Tschernogubow,[1] Hecht,[2] Noguchi,[3] Emery,[4] Gurd,[5] and others advocated the use of unheated human serum in the Wassermann test. Although it was shown that with unheated serums more positive results were obtained than with heated serums, none of the early workers attempted to measure the loss of complement-binding power incurred by heating serums to 56 C. for 30 minutes.

In this paper I shall present the results of Wassermann tests with heated and with unheated human serums. In none of these tests were native complement and hemolytic ambocepter used. Native complement was either removed by absorption or was ignored. Each human serum was tested 4 times, unheated with 0.05 c c per test tube, heated with 0.05 c c per test tube, heated and glycerolated with 0.05 c c (0.1 c c serum-glycerol mixture) of serum per test tube and heated and glycerolated with 0.125 c c (0.25 c c serum-glycerol mixture) of serum per test tube. Antigen, complement, hemolytic ambocepter, and blood corpuscles were used as described before.[6]

TEST 1

Serums 1-30, inclusive, were tested within 24 hours after having been obtained from the patients. Nos. 1-15, inclusive, were obtained from nonsyphilitic patients, and Nos. 16-30, inclusive, from syphilitics under treatment. Each serum was divided into 3 portions, A, B, and C. Portion A was heated to 56 C. for 30 minutes. From Portion B the complement was absorbed in the following manner: Of 50% suspension of washed human blood corpuscles 0.75 c c was mixed with 0.75 c c of diluted antihuman hemolytic amboceptor

Received for publication Feb. 20, 1918.

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[1] Deutsch. med. Wchnschr., 1909, 35, p. 668.

[2] Wien. klin. Wchnschr., 1909, 22, p. 338.

[3] Jour. Exper. Med., 1909, 11, p. 392.

[4] Lancet, London, 1910, 179, p. 732.

[5] Jour. Infect. Dis., 1911, 8, p. 427.

[6] Jour. Infect. Dis., 1917, 21, p. 502.

TABLE 1

SERUM WITH COMPLEMENT ABSORBED COMPARED WITH HEATED SERUM, NONGLYCEROLATED AND GLYCEROLATED

Number	Portions A {Heated Non-Glycerolated B {Unheated Complement Absorbed C {Heated Glycerolated	Serum per Tube C C	Amboceptor per Tube Unit	Readings						Results
				Antigen Tubes			Control Tubes			
				1	2	3	1′	2′	3′	
1	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.05	1.5	+	+	±	+	+	±	Negative, —.
2	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.125	1.5	+	+	±	+	+	±	Negative, —.
3	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.125	1.5	+	+	±	+	+	±	Negative, —.
4	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.125	1.5	+	+	±	+	+	±	Negative, —.
5	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.125	1.5	+	+	±	+	+	±	Negative, —.
6	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.125	1.5	+	+	tr	+	+	tr	Negative, —.
7	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.125	1.5	+	+	±	+	+	±	Negative, —.
8	A	0.05	1	+	+	tr	+	+	tr	Negative, —.
	B	0.05	1.25	+	+	tr	+	+	tr	Negative, —.
	C	0.05	1.25	+	+	tr	+	+	tr	Negative, —.
	C	0.125	1.5	+	+	0	+	+	0	Negative, —.
9	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.125	1.5	+	+	±	+	+	±	Negative, —.
10	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.125	1.5	+	+	±	+	+	±	Negative, —.
11	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	tr	+	+	tr	Negative, —.
	C	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.125	1.5	+	+	tr	+	+	tr	Negative, —.
12	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	tr	+	+	tr	Negative, —.
	C	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.125	1.5	+	+	tr	+	+	tr	Negative, —.

TABLE 1—*Continued*

SERUM WITH COMPLEMENT ABSORBED COMPARED WITH HEATED SERUM, NONGLYCEROLATED AND GLYCEROLATED

Number	Portions A {Heated Non-Glycerolated B {Unheated Complement Absorbed C {Heated Glycerolated	Serum per Tube C C	Amboceptor per Tube Unit	Readings						Results
				Antigen Tubes			Control Tubes			
				1	2	3	1′	2′	3′	
13	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	tr	+	+	tr	Negative, —.
	C	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.125	1.5	+	+	tr	+	+	tr	Negative, —.
14	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.125	1.5	+	+	±	+	+	±	Negative, —.
15	A	0.05	1.	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.125	1.5	+	+	±	+	+	±	Negative, —.
16	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	tr	+	+	±	Weakly positive, +.
	C	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.125	1.5	+	+	0	+	+	±	Moderately positive, ++.
17	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	tr	+	+	±	Weakly positive, +.
	C	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.125	1.5	+	+	0	+	+	±	Moderately positive, ++.
18	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	0	+	+	±	Moderately positive, ++.
	C	0.05	1.25	+	+	tr	+	+	±	Weakly positive, +.
	C	0.125	1.5	+	+	0	+	+	±	Strongly positive, +++.
19	A	0.05	1	+	tr	0	+	+	±	Strongly positive, +++.
	B	0.05	1.25	0	0	0	+	+	±	+++++ Strongly positive, +++++.
	C	0.05	1.25	+	0	0	+	+	±	Strongly positive, +++++.
	C	0.125	1.5	0	0	0	+	+	±	+++++ Strongly positive, +++++.
20	A	0.05	1	+	+	tr	+	+	±	Weakly positive, +.
	B	0.05	1.25	+	0	0	+	+	±	Strongly positive, ++++.
	C	0.05	1.25	+	+	tr	+	+	±	Weakly positive, +.
	C	0.125	1.5	tr	0	0	+	+	tr	+++ Strongly positive, +++.
21	A	0.05	1	+	+	0	+	+	±	Moderately positive, ++.
	B	0.05	1.25	0	0	0	+	+	tr	++++ Strongly positive, ++++.
	C	0.05	1.25	+	±	0	+	+	±	Strongly positive, +++.
	C	0.125	1.5	0	0	0	+	+	±	+++++ Strongly positive, +++++.
22	A	0.05	1	+	±	0	+	+	±	Strongly positive, +++.
	B	0.05	1.25	0	0	0	+	+	±	+++++ Strongly positive, +++++.
	C	0.05	1.25	+	±	0	+	+	±	Strongly positive, +++.
	C	0.125	1.5	0	0	0	+	+	±	+++++ Strongly positive, +++++.
23	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	tr	+	+	±	Weakly positive, +.
	C	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.125	1.5	+	+	0	+	+	±	Moderately positive, ++.

TABLE 1—*Continued*

SERUM WITH COMPLEMENT ABSORBED COMPARED WITH HEATED SERUM, NONGLYCEROLATED AND GLYCEROLATED

Number	Portions A Heated Non-Glycerolated B Unheated Complement Absorbed C Heated Glycerolated	Serum per Tube C C	Ambo-ceptor per Tube Unit	Readings: Antigen Tubes 1	Readings: Antigen Tubes 2	Readings: Antigen Tubes 3	Readings: Control Tubes 1′	Readings: Control Tubes 2′	Readings: Control Tubes 3′	Results
24	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	tr	+	+	±	Weakly positive, +.
	C	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.125	1.5	+	+	0	+	+	±	Moderately positive, ++.
25	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	0	+	+	±	Moderately positive, ++.
	C	0.05	1.25	+	+	±?	+	+	±	Faintly positive, ±.
	C	0.125	1.5	+	±	0	+	+	±	Strongly positive, +++.
26	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	0	+	+	±	Moderately positive, ++.
	C	0.05	1.25	+	+	tr	+	+	±	Weakly positive, +.
	C	0.125	1.5	+	±	0	+	+	±	Strongly positive, +++.
27	A	0.05	1	+	+	±?	+	+	±	Faintly positive, ±.
	B	0.05	1.25	+	±	0	+	+	±	Strongly positive, +++.
	C	0.05	1.25	+	+	tr	+	+	±	Weakly positive, +.
	C	0.125	1.5	+	0	0	+	+	±	Strongly positive, +++++.
28	A	0.05	1	+	±	0	+	+	±	Strongly positive, +++.
	B	0.05	1.25	0	0	0	+	+	±	Strongly positive, ++++++. ++++++
	C	0.05	1.25	+	tr	0	+	+	±	Strongly positive, ++++.
	C	0.125	1.5	0	0	0	+	+	±	Strongly positive, ++++++. ++++++
29	A	0.05	1	+	+	tr	+	+	±	Weakly positive, +.
	B	0.05	1.25	+	±	0	+	+	±	Strongly positive, +++.
	C	0.05	1.25	+	+	tr	+	+	±	Weakly positive, +.
	C	0.125	1.5	+	tr	0	+	+	±	Strongly positive, ++++.
30	A	0.05	1	+	+	0	+	+	±	Moderately positive, ++. +++
	B	0.05	1.25	±	0	0	+	+	±	Strongly positive, +++.
	C	0.05	1.25	+	+	0	+	+	±	Moderately positive, ++. ++++
	C	0.125	1.5	tr	0	0	+	+	±	Strongly positive, ++++.

representing 3 hemolytic units. This was allowed to stand at room temperature for 30 minutes. After having stood for 30 minutes 0.3 c c of the human serum was added, the mixture was well shaken, after another 30 minutes it was well centrifuged, and the clear fluid was used in the tests. Portion C was heated to 56 C. for 30 minutes and was mixed with an equal volume of sterilized glycerol. Portion C was tested in quantities of 0.05 c c and in quantities of 0.125 c c per test tube.

Table 1 shows the results obtained with Serums 1-30, inclusive. Serums 1-15, inclusive, gave negative results with all of the 4 methods used. With Serums 16-30, inclusive, the results varied greatly. Heated, glycerolated serum used in quantities of 0.125 c c per test tube gave the strongest positive results; the unheated serum came second;

TABLE 2

WHOLE SERUM COMPARED WITH HEATED SERUM, NONGLYCEROLATED AND GLYCEROLATED

Number	Portions A Heated Non-Glycerolated B Unheated Whole Serum C Heated Glycerolated	Serum per Tube C C	Ambo-ceptor per Tube Unit	Readings						Results
				Antigen Tubes			Control Tubes			
				1	2	3	1′	2′	3′	
31	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	tr	+	+	tr	Negative, —.
	C	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.125	1.5	+	+	±	+	+	±	Negative, —.
32	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	tr	+	+	tr	Negative, —.
	C	0.05	1.25	+	+	tr	+	+	tr	Negative, —.
	C	0.125	1.5	+	+	tr	+	+	tr	Negative, —.
33	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	tr	+	+	tr	Negative, —.
	C	0.05	1.25	+	+	tr	+	+	tr	Negative, —.
	C	0.125	1.5	+	+	0	+	+	0	Negative, —.
34	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.125	1.5	+	+	±	+	+	±	Negative, —.
35	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.125	1.5	+	+	±	+	+	±	Negative, —.
36	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.125	1.5	+	+	tr	+	+	tr	Negative, —.
37	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.125	1.5	+	+	±	+	+	±	Negative, —.
38	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.125	1.5	+	+	±	+	+	±	Negative, —.
39	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.125	1.5	+	+	tr	+	+	tr	Negative, —.
40	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.125	1.5	+	+	±	+	+	±	Negative, —.
41	A	0.05	1	+	+	tr	+	+	tr	Negative, —.
	B	0.05	1.25	+	tr	0	+	+	±	Strongly positive, ++++.
	C	0.05	1.25	+	+	±?	+	+	±	Faintly positive, ±.
	C	0.125	1.5	+	0	0	+	+	±	Strongly positive, +++++.
42	A	0.05	1	+	+	tr	+	+	±	Weakly positive, +.
	B	0.05	1.25	±	0	0	+	+	±	+++ Strongly positive, +++.
	C	0.05	1.25	+	+	tr	+	+	±	Weakly positive, +.
	C	0.125	1.5	tr	0	0	+	+	±	++++ Strongly positive, ++++.

TABLE 2—*Continued*

WHOLE SERUM COMPARED WITH HEATED SERUM, NONGLYCEROLATED AND GLYCEROLATED

Number	Portions A {Heated Non-Glycerolated B {Unheated Whole Serum C {Heated Glycerolated	Serum per Tube C C	Ambo-ceptor per Tube Unit	Readings						Results
				Antigen Tubes			Control Tubes			
				1	2	3	1′	2′	3′	
43	A	0.05	1	+	+	tr	+	+	±	Weakly positive, +.
	B	0.05	1.25	+	tr	0	+	+	±	Strongly positive, ++++.
	C	0.05	1.25	+	+	tr	+	+	±	Weakly positive, +.
	C	0.125	1.5	tr	0	0	+	+	tr	+++ Strongly positive, +++.
44	A	0.05	1	+	+	tr	+	+	±	Weakly positive, +.
	B	0.05	1.25	+	+	0	+	+	±	Moderately positive, ++.
	C	0.05	1.25	+	+	tr	+	+	±	Weakly positive, +.
	C	0.125	1.5	+	±	0	+	+	±	Strongly positive, +++.
45	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.125	1.5	+	+	tr	+	+	±	Weakly positive, +.
46	A	0.05	1	+	±	0	+	+	±	Strongly positive, +++.
	B	0.05	1.25	+	±	0	+	+	±	Strongly positive, +++.
	C	0.05	1.25	+	tr	0	+	+	±	Strongly positive, ++++.
	C	0.125	1.5	tr	0	0	+	+	±	Strongly positive, ++++. ++++
47	A	0.05	1	+	+	±	+	+	±	Negative, —.
	B	0.05	1.25	+	+	±?	+	+	±	Faintly positive, ±.
	C	0.05	1.25	+	+	±	+	+	±	Negative, —.
	C	0.125	1.5	+	tr	0	+	+	tr	Strongly positive, +++.
48	A	0.05	1	+	±	0	+	+	±	Strongly positive, +++.
	B	0.05	1.25	+	tr	0	+	+	±	Strongly positive, ++++.
	C	0.05	1.25	+	tr	0	+	+	±	Strongly positive, ++++.
	C	0.125	1.5	0	0	0	+	+	±	Strongly positive, +++++. +++++
49	A	0.05	1	+	+	0	+	+	±	Moderately positive, ++.
	B	0.05	1.25	±	0	0	+	+	±	+++ Strongly positive, +++.
	C	0.05	1.25	+	±	0	+	+	±	Strongly positive, +++.
	C	0.125	1.5	0	0	0	+	+	±	+++++ Strongly positive, +++++.
50	A	0.05	1	0	0	0	+	+	±	+++++ Strongly positive, +++++.
	B	0.05	1.25	0	0	0	+	+	±	+++++ Strongly positive, +++++.
	C	0.05	1.25	0	0	0	+	+	±	+++++ Strongly positive, +++++.
	C	0.125	1.5	0	0	0	+	+	±	+++++ Strongly positive, +++++.

Explanation.—+ means complete hemolysis; ±, hemolysis between 50% and 100%; tr (trace), hemolysis less than 60%; 0, no hemolysis.

heated, glycerolated serum used in quantities of 0.05 c c per test tube came third, and the weakest reactions were obtained with heated, nonglycerolated serum. A strongly positive and a negative result was obtained with Serum 25.

TEST 2

Serums 31-50, inclusive, were tested within 24 hours after having been obtained from the patients. Each serum was divided into three portions, A, B, and C. Portion A was heated to 56 C. for 30 minutes, Portion B was used whole, and Portion C was heated to 56 C. for 30 minutes and was mixed with an equal volume of sterilized glycerol. Portions A and B were used in quantities of 0.05 c c per test tube, and Portion C was used in quantities of 0.05 c c and 0.125 c c per test tube. Serums 31-40, inclusive, were obtained from nonsyphilitic persons, and Serums 41-50, inclusive, from syphilitics under treatment.

The results obtained in Test 2 are shown in Table 2. Serums 31-40, inclusive, gave negative results with all of the 4 methods used. With Serums 41-50, inclusive, the results varied greatly. Heated, glycerolated serum used in quantities of 0.125 c c per test tube gave the strongest positive results; unheated serum came second; heated, glycerolated serum in quantities of 0.05 c c per test tube came third, and plain heated serum gave the weakest reactions. Of Serum 41, Portion A gave a negative result, Portion B gave a strongly positive result, Portion C in quantities of 0.05 c c per test tube gave a faintly positive result, and Portion C in quantities of 0.125 c c per test tube gave a stronger positive result than did Portion B in quantities of 0.05 c c per test tube.

CONCLUSIONS

Unheated serum frequently gives a much stronger positive result with the Wassermann test than does heated serum.

The use of unheated serum is not practicable because it must be tested while still fresh, and because the complement present would interfere if one wished to use large quantities of serum.

In quantities of 0.05 c c of unheated human serum per test tube the native complement was not perceptible in the tests.

Quantities of 0.125 c c of heated, glycerolated serum per test tube gave stronger positive results than did quantities of 0.05 c c of unheated serum.

Heated, glycerolated serum gave a little stronger positive results than did heated, nonglycerated serum.